DEVICE FOR DIRECTIONAL COOLING OF BIOLOGICAL MATTER

FIELD OF THE INVENTION

This invention relates to controlled freezing biological material, and more particularly to a directional freezing device adapted for that purpose.

BACKGROUND OF THE INVENTION

Transfusion of blood to someone who has suffered an injury or undergone a medical procedure resulting in blood loss is a well known and common practice. The transfused blood is typically donated by volunteers, and collected by organizations such as the International Red Cross, Magen David Adom, and private collection centers. Levels of blood donation are seasonal, dropping at certain times during the year, and are also affected by major events, such as the September 11, 2001 attacks on the United States. After such well-publicized events, blood donations tend to soar, often irrespective of need (after the September 11, 2001 attacks on the United States, for example, over half a million units were donated, while fewer than 300 were used for those injured in the attacks). Methods have been developed to store donated blood for future need.

Fresh Red Blood Cells (RBC) can be refrigerated for up to 42 days, after which they are discarded due to RBC recovery falling below 70% (for this reason, over 200,000 units of blood were discarded following the September 11 attacks). Frozen RBC units can be stored for up to 10 years.

During freezing of blood, the rate of cooling affects the morphology of the intracellular ice crystals. Maximizing the survival rate of RBCs requires careful control of the freezing process. Conventional freezing devices involve lowering the temperature of the chamber in a controlled stepped manner. The thermal

gradient within the sample is determined implicitly by the temperature of the chamber and the thermal conductivity of the materials of the sample, and is not directly controllable.

US 5,873,254 discloses a device for controlled freezing and warming of a biological sample, and freezing and thawing protocols for which the device is well suited. The device establishes a laterally varying thermal gradient and provides a mechanism for moving the sample along the thermal gradient at a controlled rate of speed. The sample is moved along the thermal gradient at a rate of speed that provides a variable cooling rate or a variable warming rate in accordance with the appropriate protocol. The device also allows continuous seeding of the sample through the freezing process at the exact freezing point of the solution. Real time monitoring and video imaging of the freezing process enable fine tuning of the thermodynamic parameters for improved control. However, the device is suited for small samples.

WO 03/056919 disclose a method for changing the temperature of a sample from an initial temperature via an intermediate temperature to a final temperature. Either the initial or the final temperatures is above the freezing point of said sample and the other is below the freezing point. The method is for changing the temperature of a sample having minimal dimension in each of two mutually perpendicular cross-sections exceeding 0.5 centimeters, and at least one of the cross-sections has an outer zone and an inner zone.

SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided an apparatus for freezing a biological sample in a container while it moves along a longitudinal axis of the apparatus. The container has a first container dimension perpendicular to the axis, a second container dimension parallel to the axis, and a container thickness. The first container dimension is defined by the maximum level which the sample may have along the dimension. The apparatus comprises

at least one set of two cooling plates with inner surfaces having a first plate dimension perpendicular to the axis, and a second plate dimension parallel to the axis. The inner surfaces define a passage therebetween whose width corresponds to the container thickness and which is no larger than the first plate dimension. The first plate dimension is at least as large as the level of the biological sample along the first container dimension during use. The apparatus further comprises a motion unit adapted for movement of the container through the passage along the axis so as to allow cooling of the sample by conduction from the inner surfaces of the plates.

An apparatus according to the present invention has several advantages. One of the advantages is that it allows for the possibility of large-scale freezing of biological samples using a directional freezing technique.

The apparatus is designed so that the inner surfaces of the plates are parallel to side walls of the containers, and preferably so as to facilitate the movement of the container through the passage. This includes, but is not limited to, ensuring a constant cross section throughout the length of the passage, and providing smooth inner surfaces.

In order to facilitate cooling of the biological sample, each cooling plate may comprise at least one channel adapted for flow of a cryogenic fluid, such as liquid nitrogen, essentially along both plate dimensions. However, the cooling may be accomplished by any suitable means, including, but not limited to, externally mounted tubes adapted for flow of cryogenic fluid therethrough. In addition, there may be provided a heating arrangement, such as electric resistance heaters, in order to better control the cooling process. The apparatus may further have associated with it a feedback control system and monitoring means.

According to one embodiment of the present invention, the cooling plates are arranged in a vertical configuration in which the plates are parallel to each other, and their heights, constituting the first plate dimension, define the maximum first container dimension. According to another embodiment, the

cooling plates are arranged in a horizontal configuration, in which one plate is disposed parallel above the other, and their widths, constituting the first plate dimension, define the maximum first container dimension.

According to another aspect of the present invention, there is provided a method of cooling a biological sample. The method comprises providing an apparatus as described above, inserting therein a container containing a biological sample to be frozen, providing a predetermined temperature gradient along the axis, and moving the container through the passage along the axis.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, embodiments will now be described, by way of non-limiting examples only, with reference to the accompanying drawings, in which:

Fig. 1 is a perspective view of an apparatus according to one embodiment of the present invention;

Figs. 2A and **2B** are cross-sectional views of plates according to the present invention;

Fig. 3 is a schematic view of a feedback loop according to the present invention;

Fig. 4A is a schematic view of monitoring means according to the present invention;

Fig. 4B is a schematic view of an alternative arrangement of monitoring means according to the present invention;

Fig. 5 is a perspective view of an apparatus according to another embodiment of the present invention; and

Fig. 6 is a close-up and partially sectioned view of the apparatus of Fig. 5.

DETAILED DESCRIPTION OF THE INVENTION

Fig. 1 illustrates a freezing apparatus **10**, adapted to freeze a container **C** containing a biological sample (not shown), according to one embodiment of the present invention. The freezing apparatus **10** comprises a base **11** and a plurality of vertically oriented plates **12** mounted thereon parallel to longitudinal axis **X** of the device on both sides thereof. Inner surfaces **13** of the plates define between them a narrow passage **14** of width **W** which is throughout their length along the axis **X**. It should be noted that the width **W** may be adjustable. The plates' height **H** is greater than the width **W** of the passage, and may be as small as twice the size thereof.

There is further provided a retention device **16** adapted to grasp the container **C** and a motion unit **18** for moving the retention device through the passage **14**. The motion unit **18** is adapted to move the retention device **16** at various speeds along the passage **14**. According to this embodiment, the retention mechanism comprises two vertical prongs **20**, spaced by a distance **L**, at least one of which may be movable to adjust the distance **L** to suit the container's dimension along the axis **X**. As shown, the container is received between the prongs **20** so as to thermally contact the inner surfaces **13** when inserted, to be cooled by conduction. It should be noted that hereinafter in the specification and claims, the term *contact* should be understood to mean any contact, direct contact or abutting contact, with or without intermediate agent or means.

In order to facilitate easy movement of the container **C** through the passage, while not adversely affecting the cooling, at least one of several features may be present. The container **C** and the inner surfaces **13** may be smooth, further allowing tight contact between the inner surfaces **13** and the container **C** when the container passes through the passage. The container **C** is preferably flexible, so as to allow even distribution of the sample against both inner surfaces **13**.

Alternatively, there may be two thin sheets of a thermoconductive material (not shown) extending between the prongs, or one sheet wrapped around them, defining a rectangular box-shaped void for retaining therein the container. The width of the prongs **20** of the retention device **16** is such so that it slidingly fits within the passage **14**. It should be noted that the thin sheets, if provided, serve the dual purpose of retaining the container within the retention device **16** before it is moved within the passage **14** and preventing the container from sticking to the plates **12** during freezing. The first need may be obviated by initially moving the retention device **16**, without the container **C**, into the passage **14** and then placing the container therein through the open top of the passage, or by making the container of a stiff-walled container which is adapted to maintain its shape in the absence of such thin sheets. The second need may be obviated by using a container which is adapted not to stick to the plates **12** during freezing, or by use of the freezing apparatus under conditions, such as low humidity, such that the sticking does not occur.

The plates **12** are of a thermoconducting material, preferably brass, although other suitable materials, such as, but not limited to, aluminum, may be used. The plates **12** comprise channels **24** (seen in Figs. 2A and 2B) formed therein. Upstream and downstream endpoints **23** of the channels are in sealed fluid communication with connections **22**, adapted for ingress and egress of a cryogenic fluid such as liquid nitrogen (LN). A first connection of the pair serves as an inlet for the cryogenic fluid and a second connection serves as an outlet. It should be noted that each plate **12** may comprise one or more channels **24**. The plates **12** further comprise heating elements **26**, which are typically electrical resistance heaters, adapted to further control the temperature, as described in more detail below.

Since the plates **12** are adapted to cool by conduction, it is important to maintain direct contact between them and the container **C**. There may therefore be provided a mechanism for ensuring that the plates maintain a tight contact

even during expanding and contracting of the biological sample within a flexible container. The plates **12**, at least on one side, may be mounted on springs in order to automatically adjust to the varying width of the container **C** and maintain direct contact. Alternatively, the plates **12** themselves may be constructed so that the inner surfaces **13** thereof are biased on springs toward the direction of the passage **14**.

As illustrated in Fig. 3, the freezing apparatus **10** further comprises a feedback loop **28**. Typically, the feedback loop ensures that an appropriate temperature gradient is provided by the freezing apparatus. The feedback loop **28** comprises temperature sensors **30**, which are preferably thermocouples, disposed in strategic locations along the inner surfaces **13** of the plates **12**. The temperature sensors **30** are attached, either directly or indirectly, to a processing unit **32**. The processing unit **32** is preferably a PLC, but may be any suitable device, such as a computer having control software. The processing unit **32** is preferably capable of controlling the flow of the cryogenic fluid by independently controlling several cryogenic valves **33** in order to produce a preferred temperature gradient along the length of the passage **114**. It should be noted that this may be a non-uniform gradient, i.e., where the temperature change per linear distance changes over the length of the axis, or a zero gradient, i.e., where the temperature is constant along the entire length of the axis. The processing unit **32** is also preferably adapted to control the pressure in the supply tank of cryogenic fluid, the heating elements **26**, and may be adapted to control the operation of motion unit **18**. It may further be adapted to be preloaded with information concerning the size of the sample, desired end temperature, and one or more specific freezing protocols, which may vary depending on the type of biological sample being frozen and/or its intended use. The processing unit **32** may be provided with a display **34** adapted for displaying relevant parameters thereon. The display **34** may be a touch-sensitive screen. The processing unit **32** is also preferably adapted for documenting the freezing process.

As seen in Fig. 4A, the plates may be arranged with gaps **40**. In such a case, the freezing apparatus **10** may further comprise monitoring means, which may be disposed in the gaps **40**. However, it should be noted that the monitoring means may be arranged above the passage **14**, as seen in Fig. 4B, without including gaps. However, the first arrangement has the advantage that the center of the sample is more easily monitored, which, due to the absence of boundary effects inherent with viewing the top edge of the container, is more representative of the condition of the majority of the sample during freezing. The image from the video camera and/or the readings from the thermographs may be presented on the display **34** of the processing unit or on an independent monitor (not shown).

Freezing a biological sample using a freezing apparatus as described above has the advantage that a relatively narrow yet optionally tall container can be used, and still achieve controlled freezing of a large biological sample in a directional manner without extensive damage to cells. For example, a container with dimensions of 10 mm width × 200 mm height × 300 mm depth can be used to freeze a 600 ml sample using the freezing apparatus as seen in Fig. 1.

Figs. 5 and 6 show another embodiment of a freezing apparatus of the present invention. For the sake of clarity, elements in the freezer which are similar to elements disclosed in connection with the previous embodiment of Fig. 1 are designated with the same reference numbers shifted by 100.

A freezing apparatus **110** is adapted to freeze a container **100C** containing a biological sample. The freezing apparatus **110** according to the present embodiment comprises a base **111** and a plurality of horizontally arranged plates **112** mounted thereon parallel to an axis **100C** on both sides thereof. Upper plates **112a** are disposed opposite corresponding lower plates **112b**. Inner surfaces **113** of the plates define between them a narrow passage **114** of essentially constant height **100H**, at least during use, throughout their length along the axis **100X**. The upper plates **112a** are supported from above by springs (not shown). The springs ensure that a full contact is maintained between the upper plate **112a** and

the container. The plates' width **100W** is greater than the height **100H** of the passage, and may be as small as twice the size thereof . The container **100C** and the narrow passage **114** are smooth so that there is tight contact between the inner surfaces **113** and the container **100C** when the container passes through the passage.

There is further provided a clasp **116** adapted to grasp the container **100C** and a motion unit **118** for moving the retention device through the passage **114**. According to this embodiment, the clasp is adapted to pull the container along the axis **100X**.

Although not specifically shown in the figures with reference to the present embodiment, it should be noted that heating and cooling means, as well as feedback and monitoring means, may be provided similarly as in the first embodiment as shown in Figs. 1 through 4.

According to any one of the above embodiments, the narrow passage may be divided into three functional areas. The first area is designated as the *initial chamber*. In this area, the container is initially received, and the sidewalls of the passage **14** may be made of thermally insulating material. The initial chamber may be adapted to hold the container at a predetermined temperature. In addition, the freezing process may be initiated here by seeding. Seeding is accomplished by freezing a very small area of the container **C**, for example, with the cryogenic fluid. It should be noted that the freezing apparatus may be adapted to perform the seeding internal to the chamber, such as described, or external to the chamber, using any device suited for that purpose.

When the seeding is done externally, the container should be positioned is such a way so that the freezing is initiated at a top part of the container. This ensures that the area where seeding is accomplished is largely free of living cells. Before or upon introduction into the passage, the container is rotates so that the area where the seeding had been accomplished is at the front in the direction of motion.

The second functional area is designated as the *freezing block*. In this area, the plates **12** are designed to freeze the biological sample as described above.

The third functional area is referred to as the *collection chamber*. This area is adapted for removal of the container from the device. In addition, the biological sample may be further cooled to extremely low temperatures suitable for long-term storage. In this area, one or more of the plates **12** may be made from a thermally insulating material.

The freezing apparatus according to any of the above embodiments may be used or constructed in such a manner such that the longitudinal axis is oriented vertically. In such a setup, the gradient should be such that the temperature is lower the higher along the axis the container travels. The container therefore is moved upwardly along the axis. This is particularly important when seeding is done internally, since this orientation allows for the cells to descend in the container, and the freezing process is initiated in an area which is largely liquid and devoid of living cells. It should be noted that when the seeding is accomplished externally as described above, the gradient may be oriented so that the container is moved upwardly or downwardly along the axis.

Those skilled in the art to which this invention pertains will readily appreciate that numerous changes, variations and modifications can be made without departing from the scope of the invention mutatis mutandis. For example, the freezing apparatus is not limited to having a plurality of plates. It may be constructed using only two plates, one on each side of the narrow passage.